

**IMPACT OF AZODRIN AND *AEROMONAS VERONII* ON ENZYME INDICATORS OF FISH *CATLA CATLA* AGAINST TO IMMUNOMODULANT SILVER NANOPARTICLES**

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**ABSTRACT**

Silver nanoparticles constitute a very challenging approach for the elaboration of new antibacterial systems. Silver nanoparticles are synthesized by physical, chemical and biological modes of actions. In the present study the silver nanoparticles are synthesized by the fish scale extract of *catla catla* which is considered as a waste material and due to excitation of Surface Plasmon Resonance the synthesis of silver nanoparticles takes place. Aquatic environment by various pollutants like pesticides, discharging from various industries, induce changes in the behavioural, physiological and biochemical aspects of inhabitants, particularly fishes, which are challenging the aquaculture production. In the present study Azodrin and *A.veronii* showed increased trend in AAT and AIAT and also showed decreased trend in ALP and ACP. Silver nanoparticles acts as a immunomodulators and reduced the toxic effects of Azodrin on enzyme indicators (AAT, AIAT, ACP and ALP) of fish and also showed antibacterial activity against fish pathogen *A.veronii* and helps in the recovery of the animal.

**KEYWORDS:** Silver nanoparticles, Azodrin, *A.veronii*, Fish scale extract, Enzyme Indicators, Antibacterial activity, *Catla catla*.

**INTRODUCTION**

Aquaculture continues to be the fastest growing animal food-producing sector and can help in maintaining the socioeconomic status. Aquaculture has the ability to contribute significantly to food and nutrition security in the society. It has been reported that about 20% per capita intake of animal protein. It is highly rich source of micronutrients, minerals, proteins and essential fatty acids (Mahajan, 2011).

Direct use of silver nanoparticles in water to treat a fungal disease has been found toxic to young trout whereas a water filter coated with silver nanoparticles can prevent the fungal infections in rainbow trout fish in the fish culture. It can say here that the health of fish in aquaculture, nanotechnological applications on antibacterial surfaces in the aquaculture system, nanodelivery of veterinary products in fish food using porous nanostructures and nanosensors for detecting pathogens in the fish culture system. Thus nanomaterials have shown great potential in a wide range of the pond-ecosystem environmental nanoscale iron-manganese binary oxide was an effective sorbent for removal of arsenic (III) and arsenic (V) from both synthetic and actual field ground water of fish culture.

Silver nanoparticles often exhibit novel characteristics such as extraordinary strength, more chemical reactivity, and possessing a high electrical conductivity. Thus,

nanotechnology has become one of the most promising new approaches for pest control in the recent years (Bhattacharyya et al., 2010). Nanoparticles represent a new generation of environmental remediation technologies that could provide cost-effective solution to some of the most challenging environmental cleanup problems (Chinnamuthu and Murugesu Boopathi, 2009). Silver has been used in many applications in pure free metal or in compound form because it possesses antimicrobial activity against pathogens, yet it is non-toxic to humans (Yeo et al., 2003; Elchiguerra et al., 2005).

Silver is a soft acid, and there is a natural tendency of an acid to react with a base, in this case, a soft acid to react with a base, in this case, a soft acid to react with a soft base (Morones et al., 2005). The cells are majorly made up of sulphur and phosphorus which are soft bases. The action of these nanoparticles on the cell can cause the reaction to take place and subsequently lead to cell death. Another fact is that the DNA has sulphur and phosphorus as its major components; the nanoparticles can act on these soft bases and destroy the DNA which would definitely lead to cell death.

Fish species are sensitive to enzymatic as well as enzyme disruption which is caused due to stress by pesticides. The use of biochemical measurements in organisms as indicators of pollution, give information about the

adaptive or deleterious responses in organisms exposed to a certain amount of chemicals. Such analysis provides early warning signals before other toxicological points, including death are evident (Livingstone, 1998). Aquaculture apart from agriculture is common in India, where fish, the non-target organisms are directly exposed to pesticides used for the control of insects and pests. Environmental pollution is caused by the development of industries, technology and an informal settlement does, however, threaten many freshwater ecosystems (Favari *et al.*, 2002).

The immune system of fish is important for defense against a variety of pathogens. Insecticides may alter the function of the immune system and result in immunodepression, uncontrolled cell proliferation, and alterations of the host defense mechanisms including innate immunity and acquire immunity against pathogens (Al-Kahtani, 2011).

Organophosphates (OPs) have become the most widely used class of insecticides in the world replacing the persistent and problematic organochlorine compounds. Exposure of aquatic ecosystems to these organophosphate pesticides is difficult to assess because of their short persistence in the water column due to low solubility and rapid degradation. However, monitoring of these insecticides is important, because they are highly toxic to fish ecosystem.

Organophosphorus pesticides such as Azodrin, deltamethrin and fenvalerate are used to protect many fruit, vegetable, nut and field crops against a wide spectrum of fungal diseases and rust in order to increase agricultural production. However, they have harmful effects on aquatic environments and organisms (Santha kumar, 1999).

Toxic chemical when enters inside the body affects humans and other organisms directly or indirectly, the enzymes of biotransformation can give an indication of toxicity in a particular organ, mostly biotransforming enzymes are high molecular weight proteins, composed of chains of amino acids linked together by peptide bonds these enzymes are widely distributed throughout the body, liver is the primary biotransforming organ due to its large size and high concentration of biotransforming enzymes.

Fish disease is one of the major threats to the feasible development of aquaculture generating loss of millions of dollars annually. *Aeromonas veronii* is a Gram-negative, rod-shaped bacterium found in fresh water and in association with animals and can grow in both aerobic and anaerobic conditions and causes a diversity of diseases in both animal and human populations (Hickman-Brenner *et al.*, 1988). It can be a pathogen of humans and a beneficial symbiont of leeches. The pervasive nature of the bacteria in aquatic environments provides significant opportunity for animals, mainly fish

and amphibians to contact and ingest organisms (Seshadri, 2006).

Enzyme indicators have been explored as potential biomarkers for variety of different organisms because these parameters are highly sensitive and conserved between species and less variable. The enzyme activities tend to be more sensitive, less variable, more highly conserved between species, and often easier to measure as stress indices (Agrahari *et al.*, 2007).

Enzyme analysis is widely used for rapid detection to predict early warning of pesticide toxicity (Duta and Areids, 2003). Aspartate and alanine transaminases (AAT and A1AT), are liver specific enzymes and they are more sensitive measure of hepatotoxicity and histopathologic changes and can be assessed within a shorter time (Balint *et al.*, 1997). The phosphatases ACP and ALP are active at specific pH and are usually termed phosphomonoesterases. Number of reports on the changes in the enzyme kinetics of the organs and blood of fish exposed to toxicants (Svoboda, 2001; Velisek *et al.*, 2006; Gabriel *et al.*, 2010; Kumaran *et al.*, 2011). In some of these studies exposure to pesticides caused either a significant increase or decrease or more effect in the enzyme activities.

Many authors studied the effect of pesticides on metabolic enzymes in fish (Joshi and Desai, 1981; Jaroli and Sharma, 2005; Sreenivasan *et al.*, 2011). However very little information is available on the alterations in enzyme activities due to Azodrin in *Catla catla*. In present investigation the attempt has been made to study the effect of Azodrin, *A. veronii* and AgNPs on metabolic enzyme activities in gill, liver, kidney and muscle tissues of fish *Catla catla*.

## MATERIALS AND METHODS

### Experimental Animal

Live specimens of *Catla catla* of (28.0 ± 1.8g) were collected from AP Govt. Fish Breeding and Hatchery Centre, Kalyani dam, near Tirupati, Chittoor district and immediately transferred to transparent polypropylene tank of 500L capacity filled with filtered, well aerated and dechlorinated bore well water. The fish were fed with a commercial pelletized formulated fish feed twice a day. The water quality is maintained constantly throughout the experimental period in control medium and also in pesticide treated aquatic medium.

### Collection of tissues

After the experimental period the fish were killed by damaging the brain and severing the spinal cord between the head and trunk region using a sharp needle and the tissues viz., gill, liver, kidney and muscle were removed from its body. They were washed in ice-cold 0.33M sucrose and blotted dry and the desired amounts of tissue were weighed and used. The tissues are homogenized in 6 volumes of homogenizing buffer (50mM Tris-HCl mixed with 1.15% KCl and pH adjusted to 7.4) using

Teflon homogenizer. The resulting homogenate was centrifuged at 16,000 g for 15min in a centrifuge at -4 °C. The supernatant was decanted and stored at -20 °C in a deep freezer for until enzymatic analysis.

### Bacterial strain

Bacterial strain, *A. veronii* was obtained from Microbial Type Culture Collection and Gene Bank (MTCC) Institute of Microbial Technology, Sector 39-A, Chandigarh, India. After obtaining bacteria, it was cultured in tryptone soya broth (Himedia) for 24 h at 37°C. After incubation period, the culture was centrifuged at 800g for 15 min at 4°C. The packed cells were washed with phosphate buffered saline (PBS; pH 7.2) twice and then the required dose was prepared in PBS. The bacterial suspension was prepared to 1x10<sup>9</sup> Colony Forming Units as determined using a Neubauer haemocytometer. The selection of bacterial dose was based on earlier report (Kiran Reddy et al., 2013).

### Synthesis of Silver nanoparticles

Initially 0.787 g silver nitrate was dissolved in 100 ml distilled water. 10% of fish scale extract was mixed with silver nitrate solution in 1:9 proportions and kept at room

temperature for 72 hrs for the development of reddish brown colour was observed and the synthesised Silver nanoparticles taken for the study.

AAT and ALAT were estimated using the method of Reitman and Frankel (1957). Homogenates (10% w/v) tissue were prepared in cold 0.25 M sucrose solution and centrifuged at 3000 rpm for 15min to obtain a clear supernatant which was used as enzyme source. Values were expressed in micro moles of pyruvate formed/mg protein /h.

Alkaline phosphatase and acid phosphatases were estimated using the method developed by Kind and King (1954).

### RESULTS AND DISCUSSION

It is obvious that the control value of AAT is high in liver relative to other tissues. Among the various tissues the percent increase in the activity of AAT in more in liver (92.60%) and less in muscle (31.17%) with the following order Liver > Kidney > Gill > Muscle exposed to pesticide and *Aeromonas veronii* (Table-1).

**Table-1: Variations in Aspartate Amino transferases (AAT) activity in Gill, Liver, Kidney and Muscle tissues of fish, *Catla catla* treated with sub-lethal concentration of Azodrin, *A.veronii* and AgNps.**

Parameter	C+AZ						C+ AZ + AV			C + AZ + AV + AgNp		
	Control	3d	7d	15d	30d	45d	15d	30d	45d	15d	30d	45d
Gill	5.15 ±0.19	5.49 ±0.22	5.84 ±0.19	6.75 ±0.21	8.16 ±0.11	8.28 ±0.24	8.86 ±0.16	9.32 ±0.34	11.46 ±0.41	10.24 ±0.39	9.15 ±0.46	7.49 ±0.38
%change	—	(6.60)	(13.39)	(31.06)	(58.44)	(60.77)	(72.03)	(80.97)	(122.52)	(98.83)	(77.66)	(45.43)
Liver	6.49 ±0.25	7.52 ±0.24	8.95 ±0.12	9.28 ±0.09	10.95 ±0.11	12.50 ±0.14	13.49 ±0.29	13.88 ±0.38	15.72 ±0.39	10.78 ±0.42	9.81 ±0.56	7.65 ±0.59
% change	—	(15.87)	(37.90)	(42.98)	(68.72)	(92.60)	(107.85)	(113.86)	(142.22)	(66.10)	(51.15)	(7.65)
Kidney	5.38 ±0.16	6.15 ±0.12	7.25 ±0.21	8.28 ±0.19	8.95 ±0.11	9.75 ±0.20	10.15 ±0.16	10.86 ±0.12	12.69 ±0.14	11.39 ±0.22	9.86 ±0.19	7.38 ±0.15
%change	—	(14.31)	(34.75)	(53.90)	(66.35)	(81.22)	(88.66)	(101.85)	(135.87)	(111.71)	(83.27)	(37.17)
Muscle	5.26 ±0.15	5.46 ±0.20	5.81 ±0.15	5.95 ±0.19	6.25 ±0.21	6.90 ±0.15	7.49 ±0.16	7.86 ±0.22	9.69 ±0.24	7.46 ±0.19	7.82 ±0.26	7.09 ±0.28
% chnge	—	(3.80)	(10.45)	(13.11)	(18.82)	(31.17)	(42.39)	(49.42)	(84.22)	(41.82)	(38.02)	(34.79)

- Values are mean± SD of 6 individual observations.
- All values are significant at P< 0.05 by ANOVA.

Aspartate (AAT) and alanine aminotransferases (ALAT) are known to play strategic role in metabolising 1-aminoacids for gluconeogenesis and also function as link between carbohydrate and protein metabolism under the altered physiological, pathological and environmental stress conditions (Nicol and Rosen, 1963). A study on *Catla catla* reported that the AAT and ALAT enzyme activities were found to be increased in liver, muscle, gill and kidney tissue of fish after the fish exposure to Azodrin, in sub lethal concentrations. The study inferred

that the increased enzyme activity was due to increased utilization of amino acids for energy synthesis, in fish suffering from toxic stress and energy crisis (Naveed et al., 2010).

It is also evident from the results that ALAT shows the maximum increase was observed on day 45 in all tissues but the highest percent increase was recorded in all tissues but the highest percent increase was recorded in liver (50.95%) on day 45. Among the various tissues the percent increase of ALAT activity is followed as Liver > Muscle > Kidney > Gill treated with Azodrin and *A.veronii* (Table-2).

**Table-2: Variations in Alanine aminotransferases (AIAT) activity in Gill, Liver, Kidney and Muscle tissues of fish, *Catla catla* treated with sub-lethal concentration of Azodrin, *A.veronii* and AgNps.**

Parameter	C+AZ						C+ AZ + AV			C + AZ + AV + AgNp		
	Control	3d	7d	15d	30d	45d	15d	30d	45d	15d	30d	45d
Gill	12.65 ±0.21	13.15 ±0.11	14.25 ±0.121	16.25 ±0.14	17.11 ±0.20	17.95 ±0.19	18.42 ±0.18	18.69 ±0.26	19.32 ±0.22	18.39 ±0.21	18.09 ±0.18	17.72 ±0.16
% change	—	(3.95)	(12.64)	(28.45)	(35.25)	(41.89)	(45.61)	(47.74)	(52.72)	(45.37)	(43.00)	(40.07)
Liver	10.50 ±0.11	11.95 ±0.14	13.14 ±0.07	13.60 ±0.11	14.25 ±0.12	16.85 ±0.19	16.94 ±0.16	17.89 ±0.29	19.38 ±0.36	16.39 ±0.32	14.49 ±0.26	12.78 ±0.29
% change	—	(13.80)	(25.23)	(29.52)	(35.71)	(60.47)	(61.42)	(70.38)	(84.57)	(56.09)	(38.00)	(21.71)
Kidney	11.85 ±0.19	12.25 ±0.12	12.89 ±0.21	13.75 ±0.21	14.95 ±0.14	16.90 ±0.15	17.86 ±0.23	18.64 ±0.39	19.39 ±0.32	18.46 ±0.26	17.49 ±0.29	15.39 ±0.27
% change	—	(3.37)	(8.77)	(16.03)	(26.16)	(42.61)	(50.71)	(57.29)	(63.62)	(55.78)	(47.59)	(29.87)
Muscle	7.61 ±0.12	8.64 ±0.15	9.28 ±0.09	9.95 ±0.21	10.14 ±0.07	12.14 ±0.20	11.45 ±0.16	12.97 ±0.24	13.46 ±0.29	12.46 ±0.19	12.13 ±0.18	11.86 ±0.27
% change	—	(13.53)	(21.94)	(30.74)	(33.24)	(59.52)	(50.45)	(70.43)	(76.87)	(63.73)	(59.39)	(55.84)

- Values are mean± SD of 6 individual observations.
- All values are significant at P< 0.05 by ANOVA.

Increase in AIAT level was observed in gill and liver tissues on chronic Azodrin exposure. This is assumed as an attempt by these tissues to overcome the pesticide toxicity. AIAT in liver-specific cytoplasmic transaminase. The increased AIAT activities in tissue suggest either increased operation of transamination or increased synthesis of amino acids. This clearly indicates that stress brings about, the metabolic reorientation in the tissues by raising energy resources through transaminase systems. Similar studies have been reported by Tilak *et al.* (2004). An insignificant slight increase in AIAT level

was observed in brain tissue. Delayed neurotoxicity is expected to be reason behind this observation. Certain organophosphate esters produce a delayed neurotoxic response 7 to 14 days after acute poisoning (Baron, 1981).

The percent decrease of ALP in various tissues exposed to pesticide are followed as liver > kidney > gill > muscle. The data of ALP activity of various tissues recorded in Table- 3 showed that there is a gradual increase near to the control and the highest increase was recorded in liver (-31.22%) followed by gill (-13.76%), kidney (-13.37%) and muscle (-16.85%) treated with Azodrin and *A.veronii* (Table-3).

**Table-3: Variations in Alkaline Phosphatase (ALP) activity in Gill, Liver, Kidney and Muscle tissues of fish, *Catla catla* treated with sub-lethal concentration of Azodrin, *A.veronii* and AgNps.**

Parameter	C+ AZ						C+ AZ+ AV			C + AZ+ AV+ AgNp		
	Control	3d	7d	15d	30d	45d	15d	30d	45d	15d	30d	45d
Gill	15.62 ±0.23	14.23 ±0.98	13.32 ±0.88	11.39 ±0.49	10.44 ±0.51	10.17 ±0.59	9.84 ±0.42	9.63 ±0.62	8.24 ±0.53	10.14 ±0.62	11.83 ±0.59	13.47 ±0.66
% change	-----	(-8.89)	(-14.72)	(-27.08)	(-33.16)	(-34.89)	(-37.00)	(-38.34)	(-47.24)	(-35.08)	(-24.26)	(-13.76)
Liver	26.74 ±0.6	22.56 ±0.62	20.79 ±0.29	19.39 ±0.26	16.49 ±0.87	14.39 ±0.41	13.49 ±0.36	12.18 ±0.42	11.46 ±0.39	14.37 ±8.26	16.74 ±0.49	18.39 ±0.42
% change	-----	(-15.63)	(-22.25)	(-27.48)	(-38.33)	(-46.18)	(-49.55)	(-54.45)	(-57.14)	(-46.26)	(-37.39)	(-31.22)
Kidney	21.69 ±0.76	19.27 ±0.16	18.71 ±0.19	17.16 ±0.19	16.25 ±0.23	15.37 ±0.72	14.39 ±0.64	12.38 ±0.59	11.43 ±0.73	15.39 ±0.72	16.78 ±0.49	18.79 ±0.54
% change	-----	(-11.15)	(-13.73)	(-20.88)	(-25.08)	(-29.13)	(-33.65)	(-42.92)	(-47.30)	(-29.04)	(-22.63)	(-13.37)
Muscle	18.72 ±0.26	17.25 ±0.18	16.93 ±0.59	16.41 ±0.76	15.73 ±0.72	14.68 ±0.49	14.21 ±0.52	13.89 ±0.58	11.79 ±0.63	13.79 ±0.73	14.52 ±0.52	16.81 ±0.69
% change	-----	(-7.85)	(-9.56)	(-12.33)	(-15.97)	(-21.58)	(-24.09)	(-25.80)	(-37.01)	(-26.33)	(-22.43)	(-10.20)

- Values are mean± SD of 6 individual observations.
- All values are significant at P< 0.05 by ANOVA.

An increase of these enzyme activities in the extracellular fluid or serum is a substrate indicator of even minor cellular damage (Palanivelu et al., 2005) and indicates stress based impairment. Generally the results of AIAT, AAT, ACP and ALP may indicate degeneration of changes and hypofunction of liver as the toxicants effects on the hepatocytes are in the form of tissue damage in which cellular enzymes are released from the cells in to the blood serum.

It is observed that the ACP values in all tissues are gradually decreased when exposed to Azodrin and *A.veronii* and the maximum decrease (- 42.52%) was observed in Liver tissue on day 45. From the data it is indicating a gradual steep decrease was recorded in all tissues relative to the control and the highest decrease was recorded in liver tissue (-50.78%) as shown in Table - 4.

**Table -4: Variations in Acid phosphatase (ACP) activity in Gill, Liver, Kidney and Muscle tissues of fish, *Catla catla* treated with Sub-lethal concentration of Azodrin, *A.veronii* and AgNps.**

Parameter	C+AZ						C+ AZ+ AV			C + AZ +AV+ AgNp		
	Control	3d	7d	15d	30d	45d	15d	30d	45d	15d	30d	45d
Gill	7.25	6.96	6.28	5.96	5.32	5.14	4.89	4.63	4.83	4.84	5.22	5.66
% change	±0.89	±0.45	±0.36	±0.23	±0.35	±0.63	±0.56	±0.44	±0.44	±0.19	±0.16	±0.14
	—	(-4.00)	(-13.37)	(-17.79)	(-26.62)	(-29.10)	(-32.55)	(-36.13)	(-37.24)	(-33.24)	(-28.00)	(-21.93)
Liver	14.65	13.64	12.19	10.94	9.25	8.53	8.42	7.21	7.41	8.36	8.59	12.36
% change	±0.54	±0.16	±0.18	±0.76	±0.65	±0.38	±0.42	±0.26	±0.32	±0.042	±0.34	±0.46
	—	(-6.89)	(-16.79)	(-25.32)	(-36.86)	(-41.77)	(-42.52)	(-50.78)	(-49.41)	(-42.93)	(-41.36)	(-15.63)
Kidney	12.96	12.18	10.87	9.28	8.74	7.87	7.87	7.64	7.12	7.49	7.69	8.98
% change	±0.44	±0.26	±0.38	±0.43	±0.32	±0.48	±0.48	±0.35	±0.53	±0.68	±0.74	±0.72
	—	(-6.01)	(-16.12)	(-28.39)	(-32.56)	(-39.27)	(-41.82)	(-42.59)	(-45.06)	(-42.20)	(-40.66)	(-30.70)
Muscle	10.96	10.12	8.92	7.90	7.27	6.45	6.13	5.79	5.42	6.49	7.87	9.85
% change	±0.76	±0.67	±0.84	±0.53	±0.42	±0.87	±0.49	±0.46	±0.42	±0.38	±0.64	±0.59
	—	(-0.58)	(-18.61)	(-27.91)	(-33.61)	(-41.14)	(-44.06)	(-47.17)	(-50.54)	(-40.78)	(-28.19)	(-10.12)

- Values are mean± SD of 6 individual observations.
- All values are significant at P< 0.05 by ANOVA.

Therefore increased enzyme activities in serum of *Catla catla* is mainly due to the leakage of this enzyme from the liver cytosol in to the blood stream as a result of liver damage by pesticide and heavy metals which gives an indication of the hepatotoxic effects of toxicants. Harvey et al. (1994) concluded that the blood levels of AAT and AIAT and ACP and ALP may increase was due to the cellular damage in the liver and that high levels of this enzymes in serum or usually indicative of disease and necrosis in the liver of animals.

Enzyme analysis of organs such as gill, liver, kidney and muscle in fish can provide important information about the internal environment of the organism (Boeger et al., 2003). Enzyme activities affect various chemical and biological reactions in the body of the fish.

Exposure of *C. gariepinus* to sub lethal concentrations of pesticides altered enzyme activities and this observation agrees with Begum (2004) and Gill et al. (1991). They reported that enzyme activities were altered in fish exposed toxicants. These changes in the enzyme activities disrupt physiological and biochemical processes (Das, 2004). These biochemical changes try to maintain equilibrium in the presence of contaminants. A shift in the activities of enzymes from the control is also used as a relevant stress indicator. A major biochemical response to the effect of organophosphate pesticides in

fishes is the inhibition of a number of enzymes (Fanta et al., 2003).

In the present study fish exposed to silver nanoparticles showed the opposite trend of results obtained against to the fish treated with Azodrin and Silver nanoparticles. The results have been showing that AAT and AIAT are decreased and ALP and ACP gradually increased and the contrasting results occurred when treated with silver nanoparticles which acts as a immunomodulant and the animal tried to reach normal level to sustain in the toxic environment.

## CONCLUSION

Silver nanoparticles with antibacterial activity against fish pathogens can become an asset for fishery and aquaculture industry as a potential alternative to antibiotics. The present study indicates that silver nanoparticles had shown good antibacterial activity against fish pathogen. It is confirmed that silver nanoparticles are capable of rendering high antibacterial activity and hence has a great potential in application of nanofeeds and nanofilters in aquaculture fields to protect aquatic animals against bacterial diseases.

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